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We salute Westwood Pharmaceuticals for their contribution to the Endowment Fund and for their continued support of clinical and investigative dermatology.

D.A.N., Denver, CO.

In This Issue . . .

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Cyclosporin A, But Not Transforming Growth Factor-Beta, Inhibits Antigen Presentation by Epidermal Langerhans Cells

One of the critical components of the immune response is the presentation of specific antigens to lymphocytes. Suppression of an immune response can be achieved by inhibition of the response of lymphocytes to antigen or by interference with the presentation of antigen by antigen-presenting cells. One potent antigen-presenting cell, the Langerhans cell, is located in the most peripheral outpost of the immune system, the skin. Langerhans cell research has provided invaluable information about the mechanisms of antigen presentation. Work with this cell is now yielding fascinating clues to the mechanisms of immunosuppression. In this issue of the Journal, two papers address the Langerhans cell as a target cell for immunosuppressive agents.

Work by Dr. Louis Dubertret's group in France describes how the immunosuppressive drug cyclosporin A may inhibit the mixed epidermal cell–lymphocyte reaction. In this system the Langerhans cell serves as the stimulator cell that presents the appropriate alloantigens and thus leads to proliferation of responder lymphocytes. This type of in vitro allogeneic response would appear to be a pertinent model for testing drugs that might be useful as immunosuppressive agents in the setting of organ transplantation. Using this model, Dr. Dubertret and colleagues verified that cyclosporin inhibits this reaction, even at a low molar concentration of 10^{-7}. When either the responder lymphocytes or the stimulator epidermal cells were pretreated with cyclosporin and then thoroughly washed, partial inhibition was still seen. Similar inhibition was seen when purified Langerhans cells were pretreated with cyclosporin. Dr. Dubertret believes that this model is a useful one for testing potential immunosuppressive drugs and that it clearly shows that cyclosporin acts to suppress the immune response not only by acting upon lymphocytes but also by altering the function of antigen-presenting cells. Cyclosporin did not appear to change the amounts of interleukin-1 or prostaglandin E_2 generated by the reaction and did not appear to change the expression of class II histocompatibility antigens on Langerhans cells. Whether cyclosporin acts upon the membrane of the antigen-presenting cells or whether it specifically alters some other soluble mediators awaits further definition.

In another report Dr. Wayne Streilein and co-workers at the University of Miami used a similar in vitro model of the allogeneic response to examine the effects of cyclosporin A and the immunosuppressive cytokine, transforming growth factor-beta (TGF-B). When irradiated peripheral blood mononuclear cells were used as stimulator cells, both cyclosporin and TGF-B inhibited the reaction in a dose-dependent manner, and pretreatment of either stimulator or responder cells resulted in significant inhibition. When epidermal cell suspensions containing Langerhans cells were used as the stimulator cells, cyclosporin inhibited the reaction, whether the epidermal cells were fresh or had been cultured for three days. Preincubation of TGF-B with cultured cells also inhibited stimulation. However, when TGF-B was preincubated with freshly isolated cells, no inhibition was seen, and there was even slight enhancement of the response. This differential susceptibility to TGF-B has several implications: it may mean that cyclosporin and TGF-B alter the function of antigen-presenting cells in different ways, and it supports the previous observation of Dr. Streilein and others that Langerhans cells *in situ* and freshly isolated cells differ significantly from those that have been cultured. Dr. Streilein proposes that the differential effect of TGF-B on antigen-presenting cells may offer clues regarding a role of this cytokine in antigen presentation within the normal epidermis and regarding another potential immunosuppressive agent that might be useful in therapy of diseases such as psoriasis.

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Expression of Loricrin, a Major Component of the Cornified Cell Envelope, Is Modulated at the Transcriptional Level by Calcium, Cell Density and Retinoic Acid

Perhaps the major protein constituent of the cornified cell envelope is a cationic protein known as loricrin. In the cornified envelope this protein is cross-linked by transglutaminase to form a protein meshwork that plays an important structural role in this end-product of epidermal differentiation. Because loricrin is not expressed until late in epidermal differentiation, an important question is what factors control the synthesis of this protein by keratinocytes. In addressing this question, one might first examine what factors modulate the transcription of the loricrin gene into messenger RNA, an important first step in protein synthesis.

An elegant paper in this issue of the Journal, by Dr. Daniel Hohl from Switzerland, and co-workers at NIH, in Houston, and in Heidelberg, addresses the control of loricrin gene transcription. Using cultured human keratinocytes, these investigators showed that, by immunoblot analysis, the loricrin protein was expressed in cells exposed to 0.35 mM or higher calcium and that retinoic acid addition led to decreased loricrin expression. They then examined the level of messenger RNA for loricrin by Northern blot analysis, using a cloned DNA probe that binds to messenger RNA for loricrin. Results showed that the messenger RNA for loricrin was stimulated by increasing the calcium concentration in the cultures, that the transcription increased with increasing density of the cultures, and that addition of retinoic acid or retinol led to inhibition of loricrin gene transcription. Similar changes in messenger RNA for filaggrin, another differentiation product of the epidermis that is not part of the cornified envelope, were seen with changes in calcium and cell density and after addition of retinoic acid or retinol. Dr. Hohl points out that this study shows that loricrin and filaggrin are coordinately expressed *in vitro* and that the transcription of genes for these proteins seems to be controlled by factors different from that of certain other epidermal proteins. Although structures similar to cornified envelopes may be seen in keratinocytes without loricrin synthesis, the cornified envelope may not be mature and fully functional until loricrin is synthesized and then cross-linked. Interference with this process may be one of the major ways in which retinoic acid alters epidermal differentiation.

Epidermal Growth Factor Receptor Expression Is Increased by Insulin-Like Growth Factor I

Control of the proliferation of keratinocytes appears to be regulated by at least three peptide growth factors that act through specific receptors: epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), also known as somatomedin C, and fibroblast growth factor (FGF). Because keratinocytes do not have receptors for platelet-derived growth factor (PDGF), this factor, recognized as a major growth factor needed for fibroblasts, is thought not to be relevant to the growth of keratinocytes. A high level of insulin may serve as a substitute for IGF-I: once the insulin receptor is saturated, insulin will bind with low affinity to the IGF-I receptor, thus supporting cell proliferation in the same manner as IGF-I. How these different keratinocyte growth factors may interact and what their importance in vivo may be remain areas of intense investigation.

Dr. James G. Krueger and colleagues at the Rockefeller University believe that these keratinocyte growth factors and their interactions with their specific receptors are important in normal growth of the epidermis and may be pertinent to diseases such as psoriasis. In this issue of the Journal, they report results of a study of how IGF-I may act synergistically with EGF to control keratinocyte proliferation. Using a keratinocyte culture system in which attachment and spreading of cells in defined medium is needed prior to proliferation, they showed that neither EGF nor IGF-I was sufficient to cause keratinocyte proliferation. However, the combination of EGF and IGF-I, or high levels of insulin in place of IGF-I, caused vigorous growth of the keratinocyte cultures. In order to pursue the apparent synergy of these two factors, these researchers then examined the binding of radiolabeled EGF to keratinocytes at different concentrations of IGF-I or insulin. They were able to demonstrate that IGF-I or insulin led to a dose-dependent increase in EGF binding to keratinocytes and that this was due to an increase in the number of EGF receptors per cell. When the distribution of receptors for IGF-I and EGF in sections of normal epidermis was examined with use of monoclonal antibodies in an immunoperoxidase technique, the EGF receptors were found in all viable layers of the epidermis, whereas the IGF-I receptors were confined to the basal cell layer, where proliferative cells normally reside.

These results are obviously pertinent to understanding the growth of keratinocyte cultures and may add to our understanding of the regulation of keratinocyte growth in normal and abnormal epidermis. The IGF-I receptor appears to be confined to the proliferative cells of the epidermis, at an anatomic site where it may interact with IGF-I or insulin from the plasma or with IGF-I produced by fibroblasts. If IGF-I interaction with its receptor leads to an increase in EGF receptors on keratinocytes, these EGF receptors may then bind, not to EGF, but to transforming growth factor alpha (TGF-a), which may be produced by the keratinocytes themselves. This complex interaction of growth factors and receptors is not yet understood to the point that the in vivo significance is clear. However, because previous studies have shown that, in psoriatic epidermis, EGF receptors appear to be up-regulated and IGF-I receptors are present in suprabasilar areas, Dr. Krueger is pursuing the hypothesis that the transmodulation of the EGF receptor by IGF-I is relevant to the hyperproliferation of psoriatic epithelium. Perhaps we will later understand that this synergistic effect of EGF and IGF-I plays a critical role in the proliferative "explosion" of keratinocytes seen in psoriasis.